IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : **10/628,792**
Applicants : **Jon A. Wolff et al.**
Filed : **07/28/2003**
Art Unit : **1654**
Examiner : **Ha, Julie**
Docket No. : **Mirus.040.01**
For: **Delivery of Molecules and Complexes to Mammalian Cells In Vivo**

Commissioner of Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132

Dear Sir:

We, Vladimir Subbotin, Julia Hegge, and James Hagstrom, hereby declare as follows:

1. Vladimir Subbotin has an MD/PhD from Novosibirsk Medical School and over 35 years experience in animal pathology.

   Julia Hegge has a Bachelor's degree in Biology and Medical Technology from Edgewood College and over 20 year experience in the Medical Technology field.

   Jim Hagstrom has a PhD in Molecular biology from the Mayo Graduate School of Medicine and has over 14 years experience in the gene delivery field.

2. We are familiar with the above captioned application and with the Twist et al. (U.S. Patent 5,633,230) reference cited in the Office Action.

3. Twist et al. teach an injection of 0.25 ml into the tail vein of a mouse. Based on our experience and knowledge of mouse physiology, tail vein injection of this volume is insufficient to result in an increase in vasculature permeability.

   Increasing vessel permeability via elevated hydrostatic pressure, as taught in U.S. Application No. 10/628,792, increases the efficiency of delivery of various molecules to extravascular cells. By injecting a larger volume at a higher rate than was done in the prior art, the vessels

within a target tissue are subjected to increased hydrostatic pressure. This pressure results in the solution and its contents moving out of the vessel and to cells in the surrounding tissue.

4. We submit with this Declaration and Response further experimental material (below) illustrating the effect of volume on increasing permeability in liver vessels following tail vein injection. The experiments were performed according to the methods provided in U.S. Patent Application 10/628,792.

Venous pressure was measured during tail vein injections as follows: Animals were anesthetized with 1-2% isoflurane. A 27 gauge butterfly catheter was placed in the tail vein and secured in place with tape. The abdominal cavity was opened and the intestines were wrapped in moist gauze and exteriorized to expose the renal vein and the Inferior Vena Cava (IVC). The left renal vein and artery (near the kidney) were ligated. A catheter (polyethylene tubing size 10) for measuring intravascular pressure was inserted into the renal vein and advance into the IVC so that the tip was near the hepatic vein. This PE tubing and the IV pressure catheter were connected to a fluid filled calibrated pressure transducer system (EasyGraf, LDS Test and Instruments, Middleton, WI, USA) and the signal output was fed into a data acquisition system (Power lab, ADInstruments, Colorado Springs, Co, USA) so that pressure data could be saved for later analysis. For tail vein injections, injected fluid enters the liver primarily and this site. The intravascular pressure data was collected during the tail vein injection. After the injection, the pressure catheter was removed, the renal vein artery and vein was ligated near the IVC and the left ureter was ligated and the kidney removed. The animals were closed and allowed to recover.

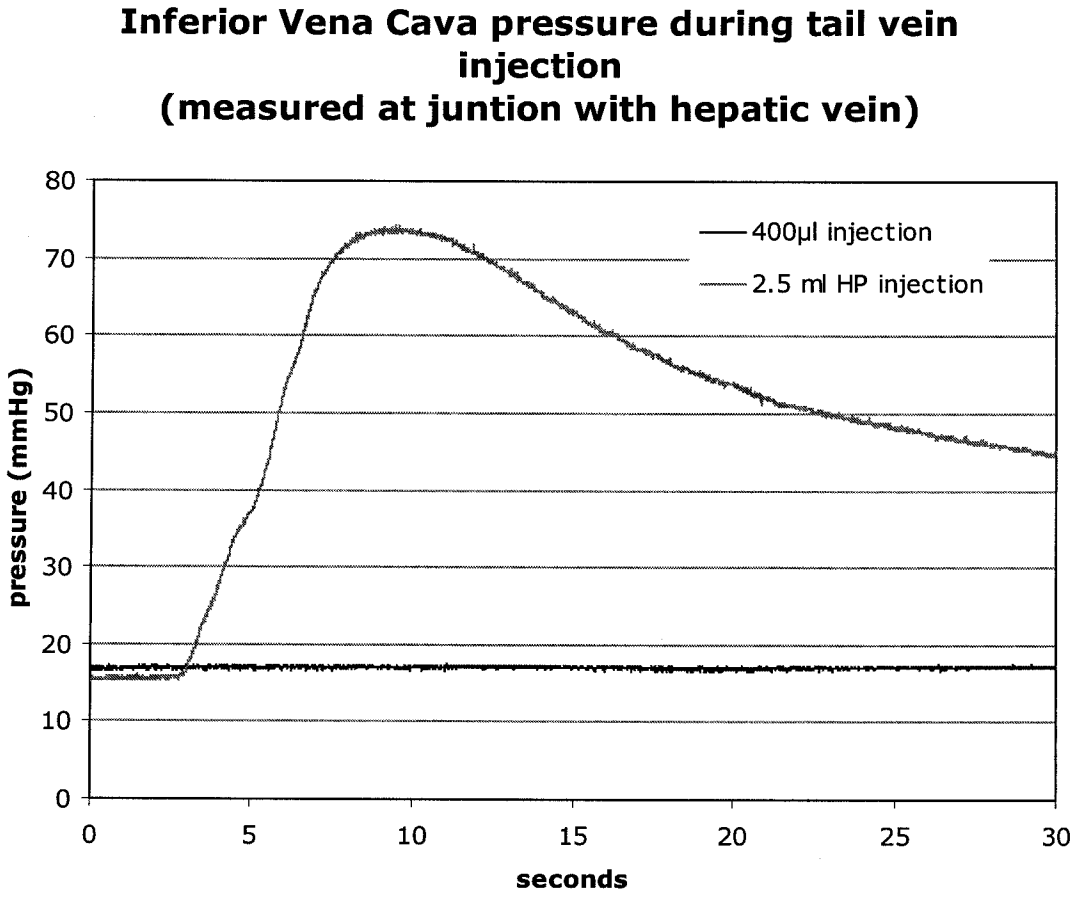
Mouse #1: Typical tail vein injection. A 400 µl solution was injected by hand in about 6-7 seconds.

Mouse #2: Mouse received a typical 400 µl injection followed by a second typical 400 µl injection.

Mouse #3: Mouse received a typical 400 µl followed by a 2.5 ml injection as described in U.S. Application No. 10/628,792.

Injection of 400 µl (60% more volume than that taught by Twist et al.) into the tail vein of a mouse resulted in no measurable increase in intravascular pressure in the Inferior Vena Cava

near the junction of the hepatic vein (see graph below). Any pressure in the hepatic vein would have been equal to or less than that measured at this location in the Inferior Vena Cava. Similarly, injection of 400 µl, followed by a second injection of 400 µl, also showed no significant increase in intravascular pressure. Conversely, injection of a larger volume, as described in U.S. Application No. 10/628,792 resulted in a greater than 4.5× increase in intravascular hydrostatic pressure near the liver. All injections were initiated at about the 3 second mark, which corresponds to the start of the rise in pressure measured for the larger volume injection.



I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

V. Subbotin 04/21/08

Dr. Vladimir Subbotin date

Julia Hegge 4/21/08

Julia Hegge date

Jan Hagstrom 4/21/08

Dr. James Hagstrom date